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# COMPARATIVE VALUE OF METHODS OF PREPARING POLLEN ANTIGEN

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The methods used to prepare pollen extract have not been at all uniform. Noon[1] extracted the pollen with distilled water, aided by repeated freezing and thawing, according to Dunbar's original method. Freeman[2] adopted the same procedure. Clowes[3] precipitated the pollen with acetone and extracted in water. Lowdermilk[4] extracted the pollen in physiologic salt solution with 0.5% phenol. Koessler[5] employed 8.5% salt solution for the extract which he sometimes precipitated with 10 times its volume of 95% alcohol. Goodale[6] prepared extracts by soaking the pollen in 15% alcohol.

In view of the diversity of methods used, it seemed advisable to investigate the relative merits of each, in order to determine the most efficient method of extraction. Accordingly, on July 16, 1915, we prepared a series of pollen antigens by various methods.

## Preparation of Antigens

A mixture was made of equal parts by weight of the dried pollens of timothy, red top, June grass, orchard grass, rye, sorrel dock, daisy, maize, ragweed, and goldenrod. A definite quantity of this mixture was extracted in different solvents, using the technic described in a previous article.[7] In each case, the proportions used gave a dilution such that 1 c.c. was equivalent to approximately 14,000 units of pollen. The pollen unit was established by Noon as the equivalent of one millionth gram of pollen.

*A*.—1.0156 gm. of the pollen mixture were extracted in 72.6 c.c. of a diluent, consisting of 66⅔% glycerol and 33⅓% saturated sodium chlorid solution.

*B*.—To 10 c.c. of Antigen A was added 0.054 c.c. of 92% phenol, making it 0.5% phenol.

*C*.—1.008 gm. of the pollen mixture were extracted in 72 c.c. of 66⅔% glycerol.

*D*.—To 10 c.c. of Antigen C was added 0.054 c.c. of 92% phenol, making it 0.5% phenol.

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[1] Lancet, 1911, 1, p. 1572.

[2] Ibid., 1911, 2, p. 814.

[3] Pro. Soc. Exper. Biol. and Med., 1913, 10, p. 70.

[4] Jour. Am. Med. Assn., 1914, 63, p. 141.

[5] Forchheimer's Therapeusis of Internal Diseases, 1914, 5, p. 671.

[6] Boston Med. and Surg. Jour., 1915, 173, p. 42.

[7] Jour. Infect. Dis., 1917, 21, p. 387.

*E.*—0.5203 gm. of the pollen mixture was extracted in 37.2 c.c. of 9% sodium chlorid solution.

*F.*—0.5434 gm. of the pollen mixture was extracted in 38.8 c.c. of distilled water.

*G.*—1.0064 gm. of pollen mixture were extracted in 71.9 c.c. of 15% alcohol.

## Complement-Fixation Tests

The 7 antigens were each subjected to the complement-fixation test on July 19, 1915, in order to determine the relative antigenic power of the extracts. The antigens were titrated against known positive antipollen serums, obtained by immunizing rabbits with an increasing number of units of pollen. The same technic was employed as that adopted by the laboratories of the Department of Health of New York City for all complement-fixation titrations. The technic is indicated in Table 1, which shows the results of titrating Antigen A.

Table 1 shows that 0.000020 gm. of pollen is the antigen unit (smallest amount that gives complete fixation). Since the pollen unit is 0.000001 gm. of pollen, it follows from this table that 1 pollen unit is equivalent to 1/20 of a unit of Antigen A. This table also shows that the antigen caused complete fixation of complement in a dilution of 1:500 (0.20 c.c. of 1:100).

TABLE 1

Titration of Antigen A (Glycerol-Salt Extract)*†

| Tube | Immune Serum, C.c. | Antigen | | 10% Complement, C.c. | 0.9% Salt, C.c. | Sensitized Erythrocyte Suspension, C.c. | Results |
|---|---|---|---|---|---|---|---|
| | | 1:100 C.c. | Grams of Pollen | | | | |
| 1 | 0.01 | 0.25 | .000025 | 0.1 | 0.0 | 0.2 | ++++ |
| 2 | 0.01 | 0.20 | .000020 | 0.1 | 0.0 | 0.2 | ++++ |
| 3 | 0.01 | 0.15 | .000015 | 0.1 | 0.05 | 0.2 | +++ |
| 4 | 0.01 | 0.10 | .000010 | 0.1 | 0.10 | 0.2 | ++ |
| 5 | 0.01 | 0.05 | .000005 | 0.1 | 0.15 | 0.2 | — |
| 6 | 0.01 | 0.025 | .0000025 | 0.1 | 0.20 | 0.2 | — |
| 7 | 0.0 | 0.4 | | 0.1 | 0.0 | 0.2 | — |
| 8 | 0.0 | 0.3 | | 0.1 | 0.0 | 0.2 | — |
| 9 | 0.0 | 0.2 | | 0.1 | 0.05 | 0.2 | — |
| 10 | 0.0 | 0.1 | | 0.1 | 0.10 | 0.2 | — |
| 11 | 0.0 | 0.05 | | 0.1 | 0.15 | 0.2 | — |
| 12 | 0.02 | 0.0 | | 0.1 | 0.20 | 0.2 | — |

* Mixtures of immune serum, antigen, and complement were placed in icebox for 15 hrs. before adding the suspension of sensitized erythrocytes. Results read after 1 hr. at 37 C.

† Citron's standard for the strength of a reaction is used in this table; namely, complete absence of hemolysis is indicated by a 4-plus sign (++++); faint hemolysis is shown by a 3-plus sign (+++); partial hemolysis is represented by a 2-plus sign (++); while a minus sign (—) indicates complete hemolysis.

## Results

Taking a unit of antigen as the smallest amount that gives complete fixation in the hemolytic series, the ratio of each antigen unit to a unit of pollen was found to be as follows:

One pollen unit was equivalent to:

1/20 of a unit of Antigen A
1/20 of a unit of Antigen B
1/50 of a unit of Antigen C
1/50 of a unit of Antigen D
1/25 of a unit of Antigen E
1/150 of a unit of Antigen F,

but the unit of Antigen G was so small that it could not be measured even in the lowest dilution (1:40) used in the titrations, inasmuch as it did not fix completely, but caused only partial fixation of complement.

Expressed in the highest dilutions of the antigens that gave complete fixation of complement, the results were:

Antigen A fixed completely in a dilution of 1:500
Antigen B fixed completely in a dilution of 1:500
Antigen C fixed completely in a dilution of 1:200
Antigen D fixed completely in a dilution of 1:200
Antigen E fixed completely in a dilution of 1:400
Antigen F fixed completely in a dilution of 1:65
Antigen G did not fix completely even in a dilution of 1:40.

## SUMMARY

All antigens were prepared with the same proportion of pollen and solvent, so that 1 c.c. of each extract was equivalent to 14,000 units of pollen.

The most complete extraction of the pollen was obtained by using a solvent composed of 66⅔% glycerol and 33⅓% saturated sodium chlorid. Such an extract in a dilution of 1:500 gave complete fixation of complement.

Pollen extracted in glycerol and salt produced antigens that were 25% stronger in antigenic properties than antigens prepared by extracting the pollen in salt solution only. The highest dilution of salt extracts that gave complete fixation of complement was 1:400.

Phenol played no part whatever in the extraction of the antigenic principle of the pollen; for the antigens fixed to the same degree, whether or not phenol was used as part of the solvent.

Glycerol alone did not extract all of the antigenic principle from the pollen; the glycerolated extract being only two fifths as strong as the extract prepared in glycerol and salt.

Distilled water gave an extract that was only about one eighth as strong as the glycerol and salt extract.